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Deuterium dilution as a method for determining total body water: effect of test protocol and sampling time

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Deuterium dilution for the measurement of total body water (TBW) has been conducted using varying protocols for equilibration. We measured TBW from deuterium dilution in urine samples in twenty-eight subjects using three protocols: (1) early morning dosage without breakfast, measuring deuterium in a second voiding at 4 h and 6 h; (2) early morning dosage with breakfast with the same measurement times; (3) dosage as last consumption before overnight sleep, measuring deuterium in a second voiding at 10 h. Results were compared with TBW estimates from underwater weighing (UWW). Because UWW is an indirect measure of TBW, it is used as an independent reference method in order to compare only relative discrepancies between the two methods. TBW values in the fasted state were not significantly different from those obtained in the fed state. The urinary deuterium enrichment was higher at 4 h than at 6 h (resulting TBW differences: 0·6 (SD 0·4) l). At 4 h and 6 h, differences in TBW measurements from deuterium and densitometry were positively related to the amount of TBW, indicating incomplete equilibration in larger water compartments. At 10 h no such relationship existed, indicating complete mixing of deuterium. It is concluded that 10 h equilibration is preferable to the shorter 4 h and 6 h, for the determination of TBW.

Deuterium dilution: Total body water: Underwater weighing

Water labelled with deuterium has been used for measurement of total body water (TBW) since the 1930s (Hevesey & Hofer, 1934; Moore, 1947). *In vivo* both the time needed for equilibration and the speed of isotope loss from the equilibrating mixture determine the isotope enrichment in the sampling medium. Therefore timing of dose administration and sampling may be critical. Body composition measurements with isotope dilution are usually performed in the postabsorptive state, i.e. in the early morning after an overnight fast and before any food or drink is consumed (Lukaski & Johnson, 1985), or after a small breakfast (Schoeller *et al.* 1980; Wong *et al.* 1988). In other studies subjects consume the dose at night before bedtime and the equilibration takes place overnight (Westerterp *et al.* 1988). The sampling medium can be urine, saliva or blood serum. It has been reported that deuterium in body water rapidly equilibrates with these media (Schloerb *et al.* 1950; Mendez *et al.* 1970; Wong *et al.* 1988). However, erroneous results may show up in urine samples if equilibration time is too short (Schoeller *et al.* 1980). The isotope dilution spaces calculated from deuterium enrichment in urine with 3, 4, 5 and 6 h post-dose samples were within 3% of each other (Wong *et al.* 1989). It is assumed that isotopic equilibrium for deuterium (Wong *et al.* 1988) and ^{18}O (Schoeller *et al.* 1980, 1982) with body water has been reached in 3 h. On the other hand it has been demonstrated that 100% equilibration of deuterium in urine had not been achieved in several subjects after a 6 h interval (Wong *et al.* 1989).

To our knowledge no validation has been carried out on the optimal equilibration. The present paper evaluates the accuracy of TBW determinations from urine samples assessed with equilibration intervals of up to 10 h. Results will be compared with TBW estimates from underwater weighing (TBW_{UWW}).

METHODS

Subjects

Fifteen adult females, aged 21–42 years, and thirteen adult males, aged 20–45 years, volunteered to participate in this study (Table 1). They were in good health and were not selected by any criteria relating to their body composition.

Deuterium dilution

Subjects received an orally administered dose of D_2O of 0·1 g/l TBW. TBW was estimated from the formula of Deurenberg *et al.* (1991). The appropriate amount of D_2O (99·8%; Akademie der Wissenschaften, Leipzig, Germany) was weighed out and diluted with tap water to 0·075 l for intake. The dose bottle was washed out and the rinse water was also ingested by the subject to ensure that all deuterium was consumed. D_2O enrichment in the body fluid was measured in urine. In all cases the second voiding of urine was taken to avoid dilution of labelled urine in the bladder. Background samples were taken immediately before dose administration. TBW was measured three times with 1-week intervals using different protocols (Fig. 1):

Expt 1: dose given after an overnight fast at 08.30 hours and no food or drink until final sample. Urine sampling from the second voiding at 12.30 and 14.30 hours (first voiding 11.00–11.30 hours).

Expt 2: dose given at 08.30 hours, but no food or drink restrictions before dose administration. Urine sampling from the second voiding at 12.30 and 14.30 hours (first voiding 11.00–11.30 hours).

Expt 3: dose given at night before bedtime (22.30 hours) and final sample from the second voiding next morning (08.30 hours), resulting in an equilibrium interval of 10 h (first voiding 07.00–07.30 hours). No food or drink until final sample.

To correct for isotope losses during equilibration, 1 week after each experiment a urine sample was taken from which the average elimination rate could be calculated. Background samples for Expts 2 and 3 served as final samples for Expts 1 and 2 respectively. Four subjects did not collect the final urine sample, making the number of subjects twenty-four with calculations using the extrapolated values (see below) from Expt 3.

Isotope abundances in urine were determined in duplicate with an isotope-ratio mass spectrometer (Aqua Sira, VG Isogas, Middlewich, Cheshire). Isotope excess was 80–90 ppm. Differences between duplicate measurements were < 1 ppm, indicating a relative imprecision of $\leqslant 1\%$. TBW was calculated as the deuterium dilution space divided by 1·04, correcting for exchange of the deuterium label with non-aqueous H of body solids (Schoeller *et al.* 1980).

TBW was calculated from enrichments at both 4 and 6 h in the first two experiments, and at 10 h in the third experiment. Enrichments were calculated from the deuterium concentration of enriched samples minus the deuterium concentration of the sample before dose administration. TBW was also determined by semi-log extrapolation to the time of dose administration (slope–intercept method), using the deuterium enrichments at 4 h, 6 h and 7 d (Expts 1 and 2) or 10 h and 7 d (Expt 3). Extrapolation was carried out using enrichment values relative to the concentration in the first urine sample before Expt 1.

Table 1. *Physical characteristics of the subjects*

	Mean	SD	Min.	Max.
Total (*n* 28)				
Age (years)	25	7	20	45
Body weight (kg)	65·6	9·4	49·2	81·7
Height (m)	1·77	0·11	1·60	2·00
Females (*n* 15)				
Age (years)	23	6	20	42
Body weight (kg)	60·5	6·3	49·2	69·9
Height (m)	1·70	0·05	1·60	1·79
Males (*n* 13)				
Age (years)	27	8	21	45
Body weight (kg)	71·5	9·0	58·4	81·7
Height (m)	1·85	0·09	1·65	2·00

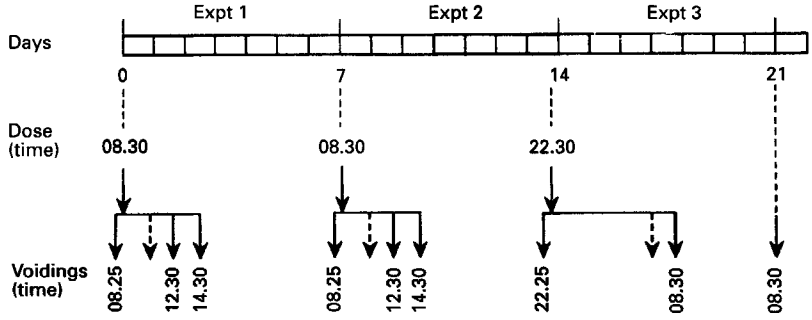

Fig. 1. Timing scheme for the deuterium experiments. For details of subjects and procedures, see Table 1 and p. 492.

Underwater weighing

Whole-body density was determined by underwater weighing (UWW) in the fasted state during week 3 of the experiments. Lung volume was measured simultaneously with the He dilution technique using a spirometer (Volugraph 2000, Mijnhardt). Percentage body fat was calculated using the equations of Siri (1956). Fat-free mass (FFM) in kg was calculated by subtracting fat mass from total body mass, and TBW was estimated by assuming that FFM has a hydration of 73% (Hunt & Groff, 1990).

Statistical analyses

The effects of feeding state (fed or fasted) and timing of the sample collection were tested by analysis of variance (ANOVA) for repeated measurements. *Post hoc* comparisons were done by paired Student's *t* tests. Results from the 10 h equilibrium experiment in the fasted state were compared with 4 h and 6 h results, also in the fasted state, using paired *t* tests. Because of multiple comparison, only P values $< 0{\cdot}01$ were accepted as statistically significant. Pearson correlation coefficients were used to test relationships between TBW calculated from isotope dilutions and from UWW. All results are expressed as mean values and standard deviations (SD).

Table 2. *Expts 1 and 2. Influence of time of sampling (4 and 6 h after dose administration and extrapolated values) and diet (fasted* v. *fed state) on values for total body water (l) measured using the deuterium dilution technique*†

(Mean values and standard deviations for twenty-eight subjects)

	Expt 1: fasted		Expt 2: fed			Mean difference		
Sampling time	Mean	SD	Mean	SD	Total mean	Mean	SD	*n*
Extrapolated	37·2	7·5	37·6**	7·6	37·4	0·4	0·7	28
4 h	37·5	7·5	38·1***	7·7	37·8	0·7	0·8	28
6 h	38·1	7·5	38·9***	7·7	38·5	0·8	0·9	28
Total mean	37·6		38·2					

Mean value was significantly different from that for Expt 1: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ by Student's paired *t* test.

† For details of subjects and procedures, see Table 1 and p. 492.

RESULTS

During Expt 2 all subjects consumed breakfast. The mean intake was 1590 (SD 770) kJ (males 2030 (SD 790) kJ, females 1210 (SD 510) kJ). The results from Expts 1 and 2 show that both feeding status and equilibration time had a significant effect on the calculated TBW compartments (ANOVA: diet $P = 0{\cdot}0001$, timing $P = 0{\cdot}0001$). TBW values in Expt 1 (fasted state) were significantly lower than those in Expt 2 (fed state) at the corresponding equilibrium time (paired *t* test, Table 2). No relationship was found between the magnitude of these differences and the energy content of the breakfast (R^2 0·12, $P = 0{\cdot}585$). There was an increase in TBW values from extrapolated values to 4 h and 6 h equilibrium time (Table 2). All differences between enrichment values and between resulting TBW values from the different equilibration times were significant in both experiments (paired *t* tests $P < 0{\cdot}001$).

The 10 h equilibrium in Expt 3 revealed a significantly higher TBW compartment (mean TBW 40·2 (SD 8·4) l) than the value from 4 h and 6 h equilibration in Expts 1 and 2 (paired *t* test $P < 0{\cdot}001$). The TBW values derived by extrapolation were not significantly different between all three experiments (paired *t* test $P > 0{\cdot}2$).

Comparison of TBW_{UWW} with $TBW_{deuterium}$ using linear regression resulted in correlation coefficients of 0·97–0·99 with all three protocols, including extrapolated enrichment values. However, the regression lines differed significantly from the line of identity (ANCOVA), except for the TBW values from 10 h equilibration (Expt 3). On average, the TBW values from deuterium dilution were 1·3–3·0 l lower than the TBW values from UWW in all experiments, except for the 10 h values from Expt 3, which revealed no significant difference.

The difference between TBW determined by the two methods plotted against the average of both measurements (Altman & Bland, 1983) showed significant associations for Expt 1 (Fig. 2, Table 3). In Expt 2 a significant association was also evident for the extrapolated value, but not for the 4 h and 6 h values ($P > 0{\cdot}06$). If the outlier (see Fig. 2, panels (A) and (B): lowest dot) is removed, all relationships in Expt 2 are significant ($P < 0{\cdot}02$). In Expt 3, neither 10 h values nor extrapolated values revealed such correlations ($P > 0{\cdot}2$). The relative biases (means of these differences) were −0·4 l and 2·2 l (10 h and 10 h extrapolated values), and the errors (standard deviation of differences) were 1·5 l and 1·3 l respectively. In conclusion, the TBW values from 10 h equilibration were independent of the amount of TBW.

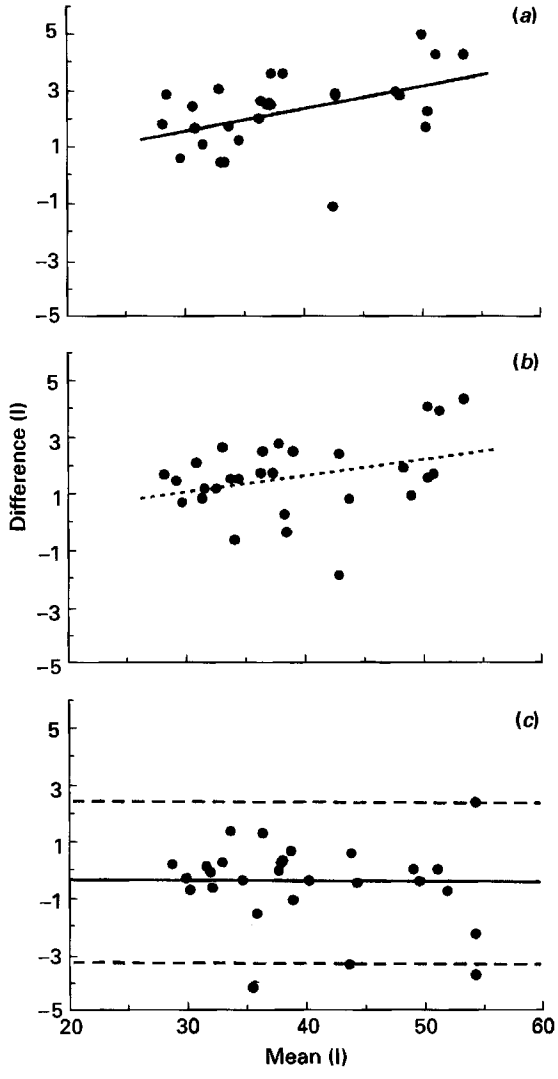

Fig. 2. Differences between total body water values derived from deuterium-dilution and those derived from underwater weighing, plotted against the mean value for both measurements. (*a*) Deuterium dilution space determined using an enrichment value of 4 h, without breakfast; (*b*) deuterium dilution space determined using an enrichment value of 4 h, with breakfast; (*c*) deuterium dilution space determined using an enrichment value of 10 h after overnight equilibration. (●), individual differences between methods; (—), relative bias; (---), upper and lower 95% limits of agreement (2 SD) between methods. In panel (*b*) the trend is indicated by dots. For details of subjects and procedures, see Table 1 and pp. 492–493. Statistical analyses: panel (*a*) R^2 0·22, $P = 0{\cdot}02$, n 28; panel (*b*) R^2 0·128, $P = 0{\cdot}06$, n 28; panel (*c*) R^2 0·03, $P = 0{\cdot}43$, n 28.

DISCUSSION

Before interpretation of the results two points have to be mentioned. First, the different experiments were not performed in a random, cross-over design. Extrapolated TBW values, however, indicated no systematic trend, allowing the following interpretations of the data. Second, the findings represented here may not apply generally depending on the physiological state of the subjects. For instance, the level of activity may influence the rates of isotope turnover or loss.

In some studies the subjects may eat before the experiment while in others the subjects

Table 3. *Correlation coefficient and* P *values of relations between differences and means of total body water estimates by deuterium dilution and underwater weighing*

Expt	Equilibrium time	R^2	*P*
1	Extrapolation	0·28	0·004
1	4 h	0·22	0·011
1	6 h	0·21	0·014
2	Extrapolation	0·21	0·016
2	4 h	0·13	0·062
2	6 h	0·12	0·073
3	Extrapolation	0·05	0·280
3	10 h	0·03	0·426

may be fasted. The present study shows that, although the difference is small, there is a significant difference between the calculated TBW compartments, lower values being obtained in the fasted state. This may be caused by a difference in equilibration time. If this is the case deuterium equilibration must be reached more quickly in the fed state, possibly because of increased intestinal transport and absorption through the intestine wall. The difference may be more pronounced when the subjects are also allowed to eat during the period of equilibration. However, without actual measurements on gastric emptying time in such experimental conditions, comments regarding isotope equilibration are speculative. It is evident that for comparative reasons it is important that all subjects within one study use the same protocol.

Since the enrichment at 6 h was significantly lower than that at 4 h it is likely that, on average, the highest enrichment values were at or before 4 h. This is in agreement with earlier studies revealing equilibrium time of deuterium by oral administration and urine samples of 1–4 h (Schloerb *et al.* 1950; Mendez *et al.* 1970; Schoeller *et al.* 1980; Wong *et al.* 1988). The higher TBW from Expt 3 with 10 h equilibration corresponds with the gradual dilution of the enrichment after approximately 4 h. Extrapolation to time of dose administration seems to correct for this phenomenon, since extrapolated values from 4 and 6 h and 10 h equilibrations result in TBW values that are not significantly different.

We compared the TBW values from deuterium dilution with the TBW values from UWW. The UWW method has not been used to obtain an absolute value of TBW, but to compare the deuterium results with TBW estimates from an independent alternative method. The most pronounced outcome of this study is that the differences between the results of the two methods in Expts 1 and 2 were related to the size of the body water compartment, indicating incomplete equilibration at the time of sampling in subjects with larger water compartments. No such relationship was observed with 10 h for equilibration (Expt 3), indicating complete mixing of deuterium in all subjects. Since enrichment values at 10 h were lower than those at 4 h, part of the deuterium is already washed out at 10 h. Thus, despite a possible small underestimation, 10 h sampling time seems to be the preferable protocol for determining TBW by deuterium dilution compared with 4 h and 6 h equilibration times.

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